BARRIER-FREE PROTEIN EXPRESSION: DEVELOPMENT OF A CELL-FREE SYSTEM WITH MINIMAL COMPONENTS

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Cell-free systems (CFS) are reactions that combine genetic material, biomolecules, and the energy required for protein synthesis without the use of whole cells. Over time, these systems have become key methods for synthetic biology applications. They have replaced and created beneficial designs for the manipulation of biological systems and have therefore proven to be a versatile tool for rapid protein synthesis, functional gene analysis, and biosensor design. However, their application remains limited by the high costs associated with key reagents, making them inaccessible in different fields of study. Studies have shown that it is possible to replace different reagents with accessible alternatives without compromising system efficiency (Guzman-Chavez et al., 2022); therefore, the search for accessible platforms is of utmost importance. In this work, the objective is to develop a robust CFS platform that allows the production of diverse proteins with distinct characteristics using the minimum requirements necessary for their synthesis. To carry out this work, it is necessary to standardize the process for obtaining low-cost cell extracts, as well as to optimize the compounds that comprise the energy buffer and the method for obtaining the DNA template. Currently, two hosts have been established for the production of viable cell extracts: Escherichia coli JM1GolddLacZ and Bacillus thuringiensis Cry-B, and different minimal formulations have been tested for the expression of pCold-GFP, pCold-ChiA74, pThur-GFP, and pThnr-GFP, which have been used as a control to evaluate the preliminary functioning of the CFS thanks to the production of a fluorescent signal.

References

1. Guzman-Chavez F, Arce A, Adhikari A, Vadhin S, Pedroza-Garcia JA, Gandini C, Ajioka JW, Molloy J, Sanchez-Nieto S, Varner JD, Federici F, Haseloff J. Constructing Cell-Free Expression Systems for Low-Cost Access. ACS Synth Biol. 2022 Mar 18;11(3):1114-1128.